VITROS Products Chemistry

INSTRUCTIONS FOR USE

TRIG

VITROS TRIG Slides

Triglyceride

Intended Use

For in vitro diagnostic use only.

VITROS TRIG Slides quantitatively measure the triclycerides (TRIG) concentration in serum and plasma.

Summary and Explanation of the Test

Triglycerides, fatty acid esters of glycerol, represent the major form of fat found in the body; their primary function is to store and provide cellular energy. The concentration of triglycerides in the plasma at any given time is a balance between the rates of entry and removal. Triglyceride concentrations in the plasma vary with age and gender. Moderate increases occur during growth and development. Triglycerides are used for the evaluation of hyperlipidemias; high concentrations may occur with hypothyroidism, nephrotic syndrome, glycogen storage diseases, and diabetes mellitus. Extremely high triglyceride concentrations are common in acute pancreatitis. [1]

Principles of the Procedure

The VITROS TRIG Slide is a dry, multilayered, analytical element coated on a polyester support. The analysis is based on an enzymatic method as described by Spayd et al. [2]

A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. The Triton X-100 surfactant in the spreading layer aids in dissociating the triglycerides from lipoprotein complexes present in the sample. The triglyceride molecules are then hydrolyzed by lipase to yield glycerol and fatty acids. Glycerol diffuses to the reagent layer, where it is phosphorylated by glycerol kinase in the presence of adenosine triphosphate (ATP). In the presence of L-α-glycerol-phosphate oxidase, L-α-glycerophosphate is then oxidized to dihydroxyacetone phosphate and hydrogen peroxide. The final reaction involves the oxidation of a leuco dye by hydrogen peroxide, catalyzed by peroxidase, to produce a dye.

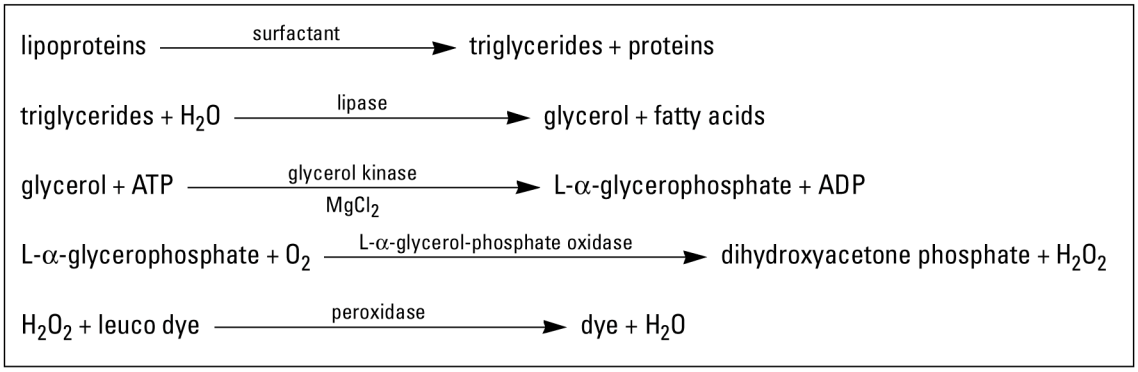
The density of the dye formed is proportional to the triglyceride concentration present in the sample and is measured by reflectance spectrophotometry.

Test Type	Wavelength	Assay Time and Temperature
Colorimetric	540 ηm	Approximately 5 minutes at 37°C

Sample Drop Volume

The volume of the sample drop depends on the format of the slide. For slides with coatings labeled 3201 and above, the sample drop volume is 5.5 μL. For all other slide formats, the sample drop volume is 10 μL.

Reaction Sequence



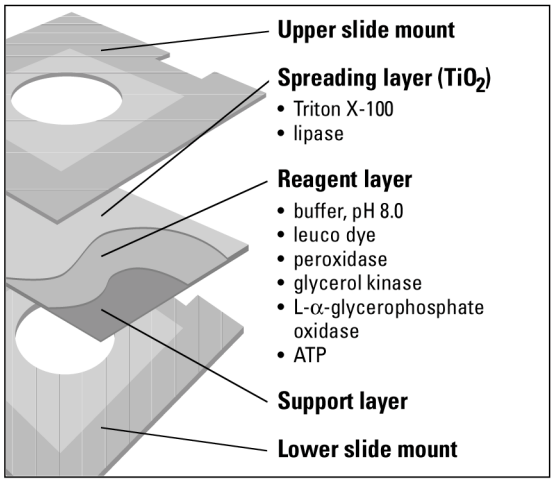
Reagents

Slide Ingredients

Reactive ingredients are lipase (*candida rugosa*, E.C.3.1.1.3); peroxidase (horseradish root, E.C.1.11.1.7); glycerol kinase (*cellulomonas sp.*, E.C.2.7.1.30); L-α-glycerol-phosphate oxidase (*pediocuccos sp.*, E.C.1.1.3.21); Triton X-100; 2-(3,5-dimethoxy-4-hydroxyphenyl)-4,5-bis(4-dimethylaminophenyl)imidazole (leuco dye); and adenosine triphosphate.

Other ingredients include pigment, binders, buffer, surfactants, stabilizers, scavenger, enzyme cofactors, dye solubilizer, and cross-linking agent.

Slide Diagram



Slide Labeling

The cartridge's outer carton is labeled with the test name, slide lot number, expiration date, and required storage temperature.

Slide Cartridge Handling

CAUTION:	**Protect the inner wrapper from damage before opening.**

- Do not drop a case of cartridges.
- Do not cut into the inner wrapper with a sharp instrument when opening the case.

Slide Storage

Unopened slide cartridges:

Store at or below -18°C (0°F).

Cartridges in the system's slide supply:

- Leave in the slide supply for no more than one week, then replace with a fresh cartridge.
- Leave in the slide supply when the system is turned off for up to two hours.
- Verify performance with control materials:
  - If the system is turned off for more than two hours
  - After reloading cartridges that have been removed from the slide supply and stored for later use

Slide Stability

VITROS TRIG Slides are stable until the expiration date on the carton when they are stored and handled as specified.

Slide Preparation

- Remove slide cartridges from storage.
- The slide cartridge must reach room temperature, 18°–28°C (64°–82°F), before it is unwrapped and loaded into the slide supply. Allow the cartridge to warm up at least 60 minutes after removing from the freezer.
- Remove the inner wrapper and immediately load into the slide supply.

NOTE:	Load the cartridges within 24 hours after they reach room temperature.

Specimen Collection and Preparation

Patient Preparation

Collect specimens from patients fasting for at least 12 and preferably 16 hours prior to the specimen draw.

Recommended Specimen Types

Serum; heparin plasma.

Serum is the specimen of choice because it is the basis for the US National Institutes of Health recommendations relating lipid levels with cardiac risk. Heparin plasma results have been reported as being within 1% of serum results.[3]

Specimens Not Recommended

EDTA plasma.

The osmotic effects of EDTA have been reported to cause an artifactual fall in lipoprotein concentration of between 3% and 5%.[4, 5] Therefore, EDTA plasma specimens for triglycerides, cholesterol, and HDL cholesterol determinations are no longer recommended.

Special Precautions

- Equipment must be soap-free and glycerol-free.
- Do not use collection tubes with glycerol-lubricated stoppers.

Specimen Collection and Preparation

- Collect specimens using standard laboratory procedures.[6, 7]
- Refer to the operator's manual section on sample handling for recommended minimum specimen volumes for your system.
- Centrifuge specimens and remove the serum from the clot within 4 hours of collection.[8]

Handling and Storage Conditions

- Handle specimens as biohazardous material.
- Handle specimens in stoppered containers to avoid contamination and evaporation.
- Storage requirements:[8]
  - Store at room temperature up to 3 days
  - Refrigerate at 2°–8°C (36°–46°F) up to 7 days
  - Freeze for storage up to 6 months
  - Avoid repeated freeze and thaw cycles

Testing Procedure

Materials Required But Not Provided

The following items are required to perform the test for TRIG:

- VITROS Chemistry Calibrator Kit 2
- Quality-control materials, such as VITROS Performance Verifiers
- For dilution, VITROS 7% BSA

Operating Instructions

Refer to the operator's manual for complete instructions on operation of your system.

Sample Dilution

If samples are grossly lipemic or show a triglycerides concentration that exceeds the system's reportable (dynamic) range, follow this procedure.

1. Dilute sample with VITROS 7% BSA.
2. Reanalyze.
3. Multiply the results by the dilution factor to obtain the original sample's triglycerides concentration.

Calibration

Required Calibrators

VITROS Chemistry Calibrator Kit 2

Calibrator Preparation, Handling, and Storage

Refer to the calibrator package insert for information about reconstitution and use of the Chemistry Calibrator Kit.

Calibration Procedure

Refer to the calibration section of your operator's manual.

When to Calibrate

- Calibrate when the slide lot number changes.
- Calibrate when critical system parts are replaced due to service or maintenance.
- If quality-control results are consistently outside acceptable limits, calibration might be required. Refer to your operator's manual for more detail.
- Calibrate when government regulations require. In the US, CLIA regulations require calibration or calibration verification at least once every six months.

Reference Methods

Calibration is traceable to the fully enzymatic method of Fossati and Prencipe.[9] The method employs a Trinder[10] reaction to detect hydrogen peroxide generated by glycerophosphate oxidase, and measures total glycerol (including free glycerol and glycerol from triglyceride hydrolysis).

Calibration Model

End-point colorimetry (described in your operator's manual).

Quality Control

Procedure Recommendations

- Handle quality-control materials as biohazardous material.
- Analyze quality-control materials in the same manner as patient samples, before or during patient sample processing.
- Analyze control materials at least once per day to verify system performance.
- Choose control levels that check the clinically relevant range.
- Refer to the quality control section in your operator's manual for additional information on quality-control procedures for VITROS Systems.
- Refer to *Internal Quality Control Testing: Principles and Definitions* for general quality-control recommendations. [11]

Quality-Control Material Selection

- VITROS Performance Verifiers are specially formulated for use with VITROS Systems.
- Other control materials may show a difference when compared with other triglycerides methods if they:
  - Depart from a true human serum/plasma matrix
  - Contain high concentrations of preservatives, stabilizers, or other nonphysiological additives
- Do not use control materials stabilized with ethylene glycol.

Quality-Control Material Preparation and Storage

Refer to the manufacturer's product literature for preparation, storage, and stability information.

Expected Values and Reporting Results

Reference Interval (Expected Values) [12]

Triglycerides Classification	Conv. Units (mg/dL)	SI Units (mmol/L)	Alternate Units (g/L)
Normal	< 150	< 1.69	< 1.50
Borderline High	150–199	1.69–2.25	1.50–1.99
High	200–499	2.26–5.63	2.00–4.99
Very High	≥ 500	≥ 5.63	≥ 5.00

Reporting Units and Unit Conversion

Conventional Units	SI Units	Alternate Units
mg/dL	mmol/L (mg/dL x 0.01129)	g/L (mg/dL x 0.01)

Limitations of the Procedure

Known Interfering Substances

- Grossly lipemic samples show a slower rate of color development than do clear serums, which results in a negative bias. These samples often contain triglyceride concentrations greater than the system's reportable (dynamic) range. See Sample Dilution under "Testing Procedure" for instructions on handling these samples.
- Free (nonesterified) glycerol in serum is measured along with the glycerol from the hydrolysis of triglycerides and diglycerides. Certain clinical conditions (e.g., diabetes mellitus and cardiac ischemia) show high endogenous free glycerol levels. Some drugs used in the treatment of lipemia also produce elevated glycerol levels. Triglyceride results from samples of such patients will not reflect actual serum triglyceride content.

Other Limitations

Some drugs and patient conditions are known to alter triglyceride concentrations in vivo. A compilation of this information is available in the literature. [13, 14]

Performance Characteristics

Reportable Range (Dynamic Range)

Conv. Units (mg/dL)	SI Units (mmol/L)	Alternate Units (g/L)
10.0–525.0	0.11–5.93	0.10–5.25

Refer to Sample Dilution under “Testing Procedure” for out-of-range samples.

Sensitivity

The lower limit of the reportable (dynamic) range is 10.0 mg/dL (0.11 mmol/L).

Precision

Precision was evaluated with quality-control materials on VITROS 250, 700, and 950 Chemistry Systems, following NCCLS Protocol EP5. [15]

These results are guidelines. Variables such as instrument maintenance, environment, slide handling/storage, control material reconstitution, and sample handling can affect the reproducibility of test results.

TRIG Precision

	Conventional Units (mg/dL)			SI Units (mmol/L)			Within Lab		
SYSTEM	Mean Conc.	Within Day SD*	Within Lab SD**	Mean Conc.	Within Day SD*	Within Lab SD**	CV%**	No. Observ.	No. Days
VITROS 250	116	1.0	1.7	1.30	0.01	0.02	1.5	80	20
	225	2.0	3.6	2.54	0.02	0.04	1.6	80	20
VITROS 700	108	0.9	1.4	1.22	0.01	0.02	1.3	91	23
	189	1.4	2.7	2.13	0.02	0.03	1.4	92	23
	230	1.8	3.4	2.60	0.02	0.04	1.5	91	23
VITROS 950	110	0.8	1.6	1.24	0.01	0.02	1.4	85	23
	232	1.7	3.6	2.62	0.02	0.04	1.6	85	23

* Within Day precision was determined using two runs/day with two to three replications.

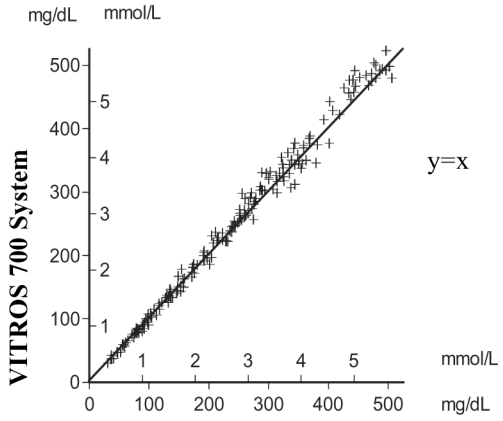
** Within Lab precision was determined using a single lot of slides and calibrating weekly.

Accuracy

The plot and table show the results of a comparison of serum specimens analyzed on the VITROS 700 System with those analyzed using the enzymatic total glycerol method of Fossati and Prencipe.[9] Testing followed NCCLS Protocol EP9. [16]

The table also shows the results of comparisons of the VITROS 250 and 950 Systems with the VITROS 700 System.

TRIG/Serom



Total Glycerol Reference Method[9]

Method Comparison (Serum)

				Conventional (mg/dL)			SI Units (mmol/L)		
	n	Slope	Correlation Coefficient	Range of Sample Concentration	Intercept	Sy.x	Range of Sample Concentration	Intercept	Sy.x
700 System vs. reference method[9]	197	1.03	0.995	31–507	-1.09	13.09	0.35–5.73	-0.01	0.15
250 System vs. 700 System	77	1.02	1.000	41–520	-2.68	3.94	0.47–5.87	-0.03	0.04
950 System vs. 700 System	117	0.99	0.999	44–510	2.07	2.36	0.50–5.76	0.02	0.03

Specificity

The following substances were tested with VITROS TRIG Slides and found not to interfere (bias < 12 mg/dL):

Compound	Concentration
Acetaminophen	5 mg/dL
Acetylsalicylic acid	30 mg/dL
Para-Aminosalicylic acid	23 mg/dL
Ascorbic acid	3 mg/dL
Bilirubin	20 mg/dL
Cholesterol	500 mg/dL
Chlorothiazide	3 mg/dL
Dextran	1000 mg/dL
Ethanol	300 mg/dL
Gentisic acid	0.5 mg/dL
Glutathione	1 mg/dL
Hemoglobin	150 mg/dL

Compound	Concentration
Hypaque	500 mg/dL
Iodide	2 meq/L
Isoniazid	0.4 mg/dL
Lactic acid	15 mg/dL
L-dopa	0.6 mg/dL
6-Mercaptopurine	1.50 mg/dL
Phospholipids	400 mg/dL
Sulfathiazole	6 mg/dL
Total protein	10 g/dL
Tyrosine	24 mg/dL
Urea nitrogen	100 mg/dL

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14. Friedman RB, Young DS. *Effects of Disease on Clinical Laboratory Tests*. Washington, D.C.: AACC Press; 1990.
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Revision History

Date of Revision:	Version:	Description:
2002APR19	1.0	New format, technically equivalent to 2001OCT18.

When this Instructions For Use is replaced, sign and date below and retain as specified by local regulations or laboratory policies, as appropriate.

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Signature Obsolete Date

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